

AMENDMENTS

This listing of claims will replace all prior versions, and listings, of claims in the application:

1. (Currently amended) A process for obtaining a population of cells enriched in viable human liver cells, including hepatic stem/progenitor cells, comprising:

(a) digesting a whole human liver or resection thereof with a proteolytic enzyme preparation to provide a digested whole human liver or resection thereof;

(b) dissociating the digested whole human liver or resection thereof to provide obtain a suspension of cells;

(c) suspending the suspension of cells in medium comprising 25% (w/v) iodixanol;

~~(d) adjusting the density of the medium in which the cells are suspended centrifuging the suspension to obtain the whereby at least two bands of cells separated by a density barrier are obtained upon centrifugation, at least one band of the at least two bands being of a lower density than another band of the at least two bands; and~~

~~(d)(e) collecting the band having density less than 1.0792 at least one band of lower density~~ to obtain a population of cells enriched in viable human liver cells, including hepatic stem/progenitor cells.

2. (Original) The process of claim 1 in which the population of cells enriched in viable human liver cells further includes functional hepatocytes.

3. (Original) The process of claim 1 in which the population of cells enriched in viable human liver cells further includes functional biliary cells.

4. (Canceled)

5. (Currently amended) The process of claim 1 in which step (a) includes:

~~(e)~~(f) perfusing the whole human liver or resection thereof with a chelation buffer;

~~(f)~~(g) digesting the whole human liver or resection thereof with an enzyme preparation comprising collagenase and at least one other proteolytic enzyme at approximately 37°C to provide a digested liver.

6. (Original) The process of claim 5 in which the enzyme preparation includes at least one neutral protease.

7. (Original) The process of claim 5 in which the enzyme preparation includes elastase.

8. (Previously presented) The process of claim 5 in which the enzyme preparation comprises both collagenase and neutral protease.

9. (Original) The process of claim 1 in which said dissociation includes mechanical dissociation.

10. (Original) The process of claim 9 in which said dissociation includes mechanical dissociation by cutting, raking, combing, or grating the liver.

11. (Currently amended) The process of claim 1 in which step (c) includes at least one of:

(h) filtering the cell suspension to remove debris and cell aggregates;

(i) collecting the resulting filtered cell suspension in a first bag;

(j) optionally determining a concentration of cells in the filtered cell suspension;

(k) adjusting, if desired, the concentration of cells to provide a starting

cell suspension;

~~_____ (l) _____ mixing an aliquot of the starting cell suspension with an equal volume of 25% iodixanol solution in a culture medium to provide a mixture; and~~

~~_____ (m) _____ subjecting at least a portion of the mixture overlaid with a predetermined volume of the culture medium to centrifugation to obtain at least one band enriched for viable human liver cells.~~

12. (Currently amended) The process of claim 1 in which step (d) includes at least one of:

(n) collecting the at least one band into a ~~container~~ collection buffer on ice;

(o) determining viability and concentration of cells;

(p) washing the cells by centrifugation and resuspension in a cryopreservation buffer to obtain a final cell suspension;

(q) subjecting the final cell suspension to controlled rate freezing to provide a frozen cell suspension; and

(r) storing the frozen cell suspension in a liquid nitrogen freezer.

13. (Currently amended) The process of claim ~~[[5]]~~ 12 in which said collection buffer comprises RPMI 1640 medium with 10% human or bovine serum.

14. (Original) The process of claim 11 in which said filtering includes passing said cell suspension through a filter cartridge.

15. (Currently amended) The process of claim ~~[[11]]~~ 1 in which said ~~culture~~ medium comprises RPMI 1640 medium lacking lacks phenol red.

16. (Original) The process of claim 11 in which said centrifugation is carried out for about 15 min at approximately 500 x g.

17. (Original) The process of claim 12 in which said container includes a collection bag.

18. (Original) The process of claim 12 in which the cryopreservation buffer comprises a mixture including Na^+ , K^+ , Ca^{2+} , Mg^{2+} , Cl^- , H_2PO_4^- , HCO_3^- , HEPES, lactobionate, sucrose, mannitol, glucose, Dextran-40, adenosine, glutathione, or combinations thereof.

19. (Original) The process of claim 18 in which the cryopreservation buffer further comprises serum and dimethylsulfoxide.

20. (Original) The process of claim 19 in which the mixture, serum and dimethylsulfoxide are present in a ratio of approximately 80:10:10 v/v/v.

21. (Original) The process of claim 19 in which the serum comprises human serum, bovine serum, or a combination thereof.

22-25. (Canceled)

26. (Original) The process of claim 1 in which the enriched population of cells includes hepatic progenitor/stem cells having a diameter in the range between 9 and 13 microns and which are positive for expression of EP-CAM, CD133, or both.

27. (Currently amended) A process for obtaining an enriched population of viable human liver cells, which population of cells comprises functional hepatocytes and hepatic stem/progenitor cells, comprising:

(a) ~~providing~~ obtaining a whole human liver or resection thereof from neonatal, pediatric, juvenile, adult, or cadaver donor;

(b) perfusing the whole human liver or resection thereof with a

chelation buffer;

(c) digesting the whole human liver or resection thereof with an enzyme preparation to provide a cell suspension;

(d) optionally, mechanically dissociating the whole liver or resection thereof to provide a cell suspension;

(e) optionally, removing debris and cell aggregates;

(f) mixing the cell suspension with an equal volume of iodixanol solution;

(g) subjecting the resulting mixture overlaid with a predetermined volume of culture medium to centrifugation to obtain at least two bands of cells separated by a density barrier, at least one band being of a lower density than another band bands; and

(h) collecting the at least one band of ~~lower~~ density less than 1.0792.

28. (Original) The process of claim 27 in which the enriched population of cells is enriched in hepatic progenitor/stem cells having a diameter in the range between about 9 and about 13 microns and which are positive for expression of EP-CAM, CD133, or both.

29-87. (Canceled)

88. (Previously presented) The process of claim 27 in which the perfusing is carried out with a chelation buffer.

89. (Previously presented) The process of claim 27 in which the enzyme preparation comprises collagenase, elastase, or both.

90. (Previously presented) The process of claim 27 in which the removing of debris and cell aggregates is carried out by passing the cell suspension through a filter cartridge.

91. (Previously presented) The process of claim 27 in which the iodixanol solution is in RPMI 1640 medium.

92. (Canceled)

93. (Currently amended) The process of claim 97 in which the density of at least one band ~~of lower density~~ is 1.0607.

94. (Currently amended) A method of obtaining an enriched population of viable human liver cells, which population of cells comprises functional hepatocytes and hepatic stem/progenitor cells, comprising:

- (a) providing a whole human liver or resection thereof;
- (b) digesting the whole human liver or resection thereof to provide a suspension of liver cells;
- (c) mixing an aliquot of the suspension of liver cells with a solution of 25% (w/v) iodixanol;
- (d) centrifuging the resulting mixture to obtain at least one band of less than 1.0792 density and enriched for viable cells; and
- (e) collecting the at least one band of viable cells.

95. (Previously presented) The method according to claim 94 in which the liver is from neonatal, pediatric, juvenile, adult, or cadaver donor.

96. (Previously presented) The method of claim 94 in which the digesting is performed with an enzyme preparation comprising collagenase, elastase or a combination thereof.

97. (Canceled)

98. (Previously presented) The method of claim 94 in which the solution of iodixanol lacks phenol red.

99. (Previously presented) The method of claim 94 further comprising overlaying the resulting mixture of liver cells and solution of iodixanol with a predetermined volume of medium lacking phenol red prior to the centrifuging step.

100. (Previously presented) The method of claim 94 in which the centrifuging is performed on a COBE™ 2991 Cell Processor.